



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. : 09/854,568
Applicants : S. BOGOCH
Filed : May 15, 2001
Title : Methods and Compositions for Stimulating the Immune System
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Art Unit : 1644
Examiner : David A. SAUNDERS
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Customer No. : 23838

Commissioner for Patents
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DECLARATION UNDER 37 CFR 1.132

S I R:

I, Samuel Bogoch, hereby declare:

1. I hold a doctorate in medicine, which was granted to me in 1951 by The University of Toronto School of Medicine. I also hold a doctorate of philosophy in biochemistry, which was granted to me in 1957 by Harvard University.
2. I conceived of the subject matter disclosed and currently claimed in U.S. patent application serial no. 09/854,568, filed May 15, 2001, and am the inventor listed therein.
3. I am knowledgeable of the art to which the invention claimed in the above-captioned patent application pertains and am likewise knowledgeable of the state of the art that existed

when the application to which the above-captioned application claims priority was filed (U.S. patent application serial no. 07/744,649, filed August 8, 1991).

4. To provide the instant declaration under 37 CFR § 1.132, I have reviewed the disclosure of the application as well as Bogoch *et al.*, Protides of Biological Fluids, 30, 337-352 (1983); U.S. Patent No. 4,840,915; Friden *et al.*, Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier, PNAS, Vol. 88, pp. 4771-4775, June 1991; Abstract, Banks *et al.*, Peptides (2002), vol. 23, no. 12, pp. 2223-2226; Abstract, Harjunpaa *et al.*, Complement activation in circulation and central nervous system after rituximab (anti-CD20) treatment of B-cell lymphoma, Leuk Lymphoma, 2001 Aug; 42(4): 731-8; Elliot *et al.*, Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy, Exp Neurol. 1996 Oct; 141(2):214-24; and Zhang *et al.*, Immunotherapy of intracranial G422 glioblastoma with dendritic cells pulsed with tumor extract or RNA, Journal of Zhejiang University SCI 2004 5(10): 1298-1303.

5. It is my understanding that a patent claim under U.S. patent law is sufficiently enabled under 35 U.S.C. § 112, paragraph 1, when the specification enables one of skill in the art to make and use the invention defined by the claim without undue experimentation. *See* MPEP § 2164.

6. Based on my review of the application, I conclude that the present application would have enabled one of skill in the art on the priority date of the application to practice the claimed method of killing glioma cancer cells *in vivo* without undue experimentation.

Production of Antimalignin Antibodies in a Subject

7. The present application teaches one of skill in the art how to produce antimalignin antibodies in a subject by administration of malignin to a subject.

8. The present application provides a detailed description of the subcutaneous administration of malignin to a subject to produce antimalignin antibody. *See* Appln. at 17-18, Example 8. The present application additionally teaches that anti malignin antibodies have been produced in mouse monoclonal form, have been produced *in vitro* in human lymphocytes

challenged with malignin, and have been isolated from human serum. Appln. at 6 *citing* Bogoch *et al.*, Lancet 1, 987 (1979)(**Attached at Tab A**), Bogoch *et al.*, Cancer Detection and Prevention 12, 312-320 (1988)(**Attached at Tab B**) and Bogoch *et al.*, Lancet 2, 141-142 (1981)(**Attached at Tab C**). I conclude, therefore, that one of skill in the art, on the priority date of the application, would have known how to produce antimalignin antibodies by administration of malignin to a subject. *See also* U.S. Patent No. 4,840,915 (Bogoch), Col. 24, Example 7.

Preferential Binding of Antimalignin Antibodies in a Subject

9. The present application teaches one of skill in the art that antimalignin antibodies bind preferentially to glioma cancer cells.

10. Example 1 of the application shows that antimalignin antibody binds preferentially to cancer cells. *See, e.g.*, Appln. at 7 (“Figure 1h and 1i show the specificity of anti-Recognin in binding selectively to two blast cells, but not to normal red and white blood cells in the same smear.”). Example 5 of the application further shows that antimalignin antibody introduced intravenously in an acceptable animal model binds preferentially *in vivo* to malignant glioma cells. *See* Appln. at 9 (“[A]nti-Recognin given intravenously has been shown to bind preferentially to malignant glioma cells in the rat brain *in vivo*.”) *citing* Bogoch *et al.*, Protides of Biological Fluids, 30, 337-352 (1983)(**Attached at Tab D**); *see also*, U.S. Patent No. 4,840,915, col. 35, line 55 – col. 36, line 18. Example 2 of the application also shows that antimalignin antibody binds human cancer tissues *in vivo* (such as adenocarcinoma and neuroblastoma). *See, e.g.* Appln. at 10 (Table, Example 2).

11. Example 5 of the application cites Bogoch *et al.*, Protides of Biological Fluids, 30, 337-352 (1983) as showing that antimalignin antibody introduced intravenously in Wistar rats bound preferentially *in vivo* to malignant glioma cells. Glioma cells were injected into the rat brains. The rats were monitored for the development of symptoms related to the establishment of brain tumors. Radioactive antimalignin antibodies were administered intravenously to the rat. After 10 days, the rats were sacrificed and the rat brain was removed. Scintillation counters demonstrated a concentration of antimalignin antibody in the rat brain where glioma cells had established a brain tumor. One of skill in the art would understand that the antimalignin

antibodies were preferentially bound to the glioma tumors that had developed in the rat brains. One of skill in the art would understand from Example 5 of the application that antimalignin antibody administered intravenously into a subject would be expected to bind preferentially in glioma tumors in the brain of the subject.

12. In view of Example 5, one of skill in the art would understand that the antimalignin antibodies of the application bound preferentially in the glioma tumors in the rat brain even in the presence of the blood-brain barrier. One of skill in the art would conclude that the antimalignin antibody crossed the blood-brain barrier, at least in some manner, since the antimalignin antibody was observed to be concentrated in the rat brain. I conclude, therefore, that in view of Example 5, one of skill in the art would expect antimalignin antibody to bind to glioma tumor cells in the brain and not to be excluded from glioma brain tumors by the blood-brain barrier.

13. One of skill in the art would expect antimalignin antibody to cross the blood-brain barrier and bind preferentially in the brain of a subject because growth of a glioma tumor would be expected to cause leakiness in the blood-brain barrier that would allow antibodies direct access across the blood-brain barrier. *See, e.g., Elliot et al., Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy, Exp Neurol. 1996 Oct; 141(2):214-24 (Attached at Tab E).* One of skill in the art would also expect the antimalignin antibody to cross the blood-brain barrier, at least in limited amounts (even without expected leakiness in the blood-brain barrier), because one of skill in the art would expect at least a limited amount of antibody to directly cross the blood-brain barrier. *See, e.g., Friden et al., Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier, PNAS, Vol. 88, pp. 4771-4775, June 1991 (“[W]e present data that indicate that this antibody and antibody-drug conjugate cross the BBB.”)(Attached at Tab F); Abstract, Banks et al., Peptides (2002), vol. 23, no. 12, pp. 2223-2226 (Attached at Tab G)(“Here, we show that antibody directed against [amyloid p protein] ApP does cross the BBB at a very low rate.”); Abstract, Harjunpaa et al., Complement activation in circulation and central nervous system after rituximab (anti-CD20) treatment of B-cell lymphoma, Luek Lymphoma 2001 Aug; 42(4): 731-8 (Attached at Tab H)(“In CSF [cerebral*

spinal fluid] the rituximab concentration increased after successive infusions, but remained below 0.55 microg/ml (compared to a Cmax of 400 microg/ml in peripheral blood.”).

14. Because antimalignin antibodies would likely be available to glioma tumor cells in the brain of a subject through leakiness in the blood-brain barrier caused by growth of the tumor and because antibodies would likely cross the blood brain barrier at least in small quantities even in the absence of leakiness, I conclude that one of skill in the art would expect antimalignin antibodies in a subject to bind to glioma tumor cells in the brain of the subject *in vivo*.

15. The application also shows that antimalignin antibody binds preferentially to human cancer tissues *in vivo*. Example 2 shows that antimalignin antibody bound preferentially in human cancer tissue removed at surgery or autopsy as compared to normal (non-cancerous) tissue. In Example 2, antimalignin antibody was measured in tissues of adenocarcinoma, transitional cell carcinoma, neuroblastoma and lymph node metastases.. *See, e.g.* Appln. at 10 (Table, Example 2). Antimalignin antibody did not bind preferentially to normal brain, muscle or liver tissue when measured in those tissues. *Id.*

16. Because the application shows (1) preferential binding of antimalignin antibody to cancer cells *in vitro*, (2) preferential binding to glioma cancer cells *in vivo* in an acceptable animal model, and (3) preferential binding in freshly excised human cancer tissue, one of skill in the art would have expected that subcutaneous administration of an effective amount of a compound sufficient to stimulate the immune system to produce antimalignin antibody would result in preferential binding to glioma cancer tissue *in vivo*.

Antimalignin Antibody Kills Glioma Cells

17. The present application teaches one of skill in the art that antimalignin antibody kills glioma cancer cells *in vivo* in serum-based culture with at least complement-dependent cytotoxicity and is active against cancer cells at picograms of antibody per cell.

18. Example 1 of the application shows that 50 µl of 10 µg/ml antimalignin antibody is cytotoxic to glioblastoma brain cancer cells growing on the wall of tissue culture flasks. *See*

Figure 1j-l, Appln. at 8 (Example 1) and at 12 (Example 5). One of skill in the art would have realized that the experiment in Example 1 demonstrates at least a complement-dependent cytotoxicity because the experiment utilized a serum-based assay (*i.e.*, containing components of the complement cascade).

19. Example 6 and Figure 2 of the application show that growth of small cell lung carcinoma cells *in vitro* was inhibited by anti-malignin antibodies in the range of picograms per cell. One of skill in the art would understand that the results provided in Example 6 and Figure 2 demonstrate cell killing activity at surprisingly low concentrations. In fact, most pharmaceutical compositions are administered at a nanogram level or even at a microgram level, which is of course is a dose that is a 1,000 to a million degree higher than the picogram dose.

One of Skill in the Art Would Expect Antimalignin Antibody
to Kill Glioma Cells in a Subject

20. I am aware of no scientific literature available at the priority date of the application that teaches or suggests antibodies shown to have complement-dependent cytotoxicity in cancer cells *in vitro* are not also cytotoxic to the same type of cancer cells *in vivo*. I conclude, therefore, that one of skill in the art would have expected antimalignin antibodies produced *in vivo* to kill glioma cancer cells *in vivo*. This conclusion would be especially evident to one of skill in the art in view of the surprisingly low concentration of antibody necessary to show cell killing activity.

21. Present day scientific literature seems to indicate that *in vitro* studies of glioma therapies translate very well to *in vivo* results. Numerous abstracts (**attached at Tab I**), show that therapies showing promise in *in vitro* tests, also show promise in *in vivo* tests. For example, in Gupta et al., Neurosurg Focus. 2006 April 15;20(4);E20, the authors report that combo therapy of tamoxifen and hypercine were able to increase the apoptosis-stimulatory potency of temozolimide in human glioblastoma multiforme cell lines, as well as in an *in vivo* xenograft mouse model. In another example, in Eller et al., Neurosurgery 2005, 56(1):155-62, the authors report that the antibody, cetuximab, was effective against EGFR-amplified glioblastoma multiforme in *in vitro* and *in vivo* models.

22. Research since the priority date of the application has confirmed the teachings of the application. Since the priority date, at least one anti-cancer antibody (Rituximab) has been shown to cross the blood-brain barrier in low concentrations compared to peripheral blood and has been shown to initiate at least minor and delayed complement activation in cerebrospinal fluid. Abstract, Harjunpaa *et al.*, Complement activation in circulation and central nervous system after rituximab (anti-CD20) treatment of B-cell lymphoma, *Leuk Lymphoma*, 2001 Aug; 42(4): 731-8. Thus, even if only small amounts of an antibody cross the blood brain barrier, since antimalignin antibody has been shown to work at picogram concentrations, one would expect antimalignin to cross the blood brain barrier at levels high enough to be effective. Thus, one of skill in the art would expect antimalignin antibody (which has been shown to bind to glioma cells in brain tumors *in vivo* and to have at least complement-dependent cytotoxicity at unexpectedly low concentrations *in vitro*) would demonstrate at least complement-dependent cytotoxicity in glioma cells *in vivo*,

23. Since the priority date of the above-captioned application, dendritic-cell based vaccines against glioma tumors have also been shown to provoke at least a cytotoxic T lymphocyte response in rats harboring intracranial glioblastomas. *See*, Zhang *et al.*, Immunotherapy of intracranial G422 glioblastoma with dendritic cells pulsed with tumor extract or RNA, *Journal of Zhejiang University SCI* 2004 5(10): 1298-1303 (**Attached at Tab J**). The dendritic-cell based immunization in Zhang *et al.* additionally significantly prolonged survival in the rats as compared to controls. *Id.* One of skill in the art would consider the success of these dendritic-cell based vaccines to confirm the teaching of the application that antimalignin antibodies shown to kill glioma cells *in vitro* and bind to glioma cells in rat brain *in vivo* would be expected to bind to glioma cells and kill glioma cells in a subject *in vivo*.

Administration of Malignin to Produce Antibodies to Kill Glioma Cells in a Subject
Does Not Require Undue Experimentation

24. Example 8 provides a detailed description of how one of skill in the art would practice the currently claimed invention. Appln. at 17-18. Example 8 teaches that malignin can be

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administered in doses of approximately 1 mg or more subcutaneously to stimulate the immune system of a subject to produce antimalignin antibodies that kill cancer cells. Appln. at 17-18.

25. I conclude the present application would have enabled one of skill in the art to administer antimalignin antibody to kill glioma cancer cells within a subject *in vivo*. I reach this conclusion because: (1) the present application teaches one of skill in the art how to subcutaneously administer malignin to produce antimalignin antibody *in vivo*, (2) the present application teaches that antimalignin antibody preferentially binds glioma cells in the brain *in vivo*, and (3) the present application teaches that antimalignin antibody kills glioma cells *in vitro* through at least complement-dependent cytotoxicity. Therefore, one of skill in the art would expect the production of antimalignin antibodies *in vivo* to preferentially bind and kill glioma cells *in vivo* with at least complement-dependent cytotoxicity and would find the present application highly suggestive of success in practicing such a method.

26. I conclude that because one of skill in the art could have followed the detailed description in Example 8 and would have found data disclosed in the application indicative of success in following the description in Example 8, one of skill in the art would not require undue experimentation to practice the invention based on the present application.

27. I, Samuel Bogoch, declare under penalty of perjury that the above statements are true and correct to the best of my knowledge, information, and belief. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: March 30, 2007

Samuel Bogoch

Dr. Samuel Bogoch